Wood decay caused by a white-rot, *Trametes villosa* (Fr.) Kreisel (Basidiomycetes, Fungi), in *Eucalyptus viminalis* Labill and *Myrcia rostrata* DC. (Myrtaceae)

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Resumo

Trametes villosa (Basidiomycetes), fungo causador de podridão branca, foi testado por 10 meses sobre a madeira de duas espécies de Myrtaceae, *Eucalyptus viminalis*, uma espécie introduzida e de *Myrcia rostrata*, espécie nativa. A degradação *in vitro* das espécies vegetais foi testada e comparada. Foram determinadas as perdas de massa e de lignina Klason. A degradação causada pelo fungo foi significativamente diferente entre as espécies vegetais. A decomposição da madeira de *M.*

rostrata foi mais rápida nos dois primeiros meses, ao contrário de *E. viminalis* onde isto ocorreu a partir do segundo mês. A perda de massa não esteve necessariamente relacionada a maiores valores de perda de lignina Klason. *Trametes villosa* atuou como deslignificador seletivo nos primeiros dois meses e não-seletivo nos meses subseqüentes em ambas as espécies vegetais. *Myrcia rostrata* foi mais resistente a *Trametes villosa* do que *E. viminalis*.

Unitermos: podridão branca, ataque fúngico, resistência vegetal, lignolítico.

Abstract

The white-rot fungus *Trametes villosa* was tested for 10 months on two Myrtaceae species, *Eucalyptus viminalis*, an exotic tree, and *Myrcia rostrata*, a native tree of Brazil. The wood decay *in vitro* caused by *T. villosa* was determined and compared between the host species. Weight and Klason lignin losses were quantified. The rate of degradation was significantly distinct between *E. viminalis* and *M. rostrata*. The degradation of the wood of *M. rostrata* was faster only during the first two months; on the other hand, *E. viminalis* presented this behavior after the second month. The weight loss was not necessarily related to high values of Klason lignin loss. *Trametes villosa* showed a selective delignification of *E. viminalis* and *M. rostrata* in the first two months and a non-selective decay in the subsequent months on both trees. The native *M. rostrata* was more resistant to *T. villosa* action than *E. viminalis*.

Key words: white rot, fungal attack, wood resistance, lignolytic.

Introduction

In the carbon cycle, lignin biodegradation is the central core, since it is the basis of natural wood decay. The annual biomass production in the world is estimated in $10x10^{10}$ ton, where $20x10^{9}$

ton correspond to lignin (Harsh and Tiwari, 1990; Jennings and Lysek, 1996). Despite being a high caloric resource, wood is stable and difficult to degrade due to its polymeric and insoluble nature. Hemicellulose and lignin together cover the cellulose, filling the spaces among the fibers, forming a net (Kirk and Fenn, 1982). Lignin physically protects polyssacharides from attack by extracellular enzymes of fungi (Aust, 1997).

Due to its high molecular weight and irregular structure, lignin can only be degraded outside the fungal cell through a specific extracellular enzyme system (Jennings and Lysek, 1996; Kotterman, 1998). These enzymes may function separately or in cooperation with each other (Leonowicz et al., 1999).

The white-rot fungi (Ascomycota and Basidiomycota) are able to degrade cellulose, hemicellulose and lignin. The delignification can be classified as simultaneous (carbohydrates and lignin are attacked more or less uniformly) or selective (hemicellulose and lignin are attacked preferentially) depending on both wood and fungi. The former type of delignification is most pronounced when weight loss is low (Worrall et al., 1997). Both processes can be influenced by either the fungi's nutritional necessities or by the wood's chemical composition and structural arrangement (Adaskaveg and Gilbertson, 1986).

The genus *Trametes* (Basidiomycetes) is one of the most aggressive white-rot fungus on dead wood, with various species (Mswaka and Magan 1998), including *Trametes villosa* (Fr.) Kreisel, with a neotropical distribution, which decays native trees in Brazil (Loguercio-Leite, 1993; Hawksworth et al., 1995).

Myrtaceae presents wide distribution in Australia and America. The native South American tree *Myrcia rostrata* DC. is commonly used as stake and firewood (Legrand and Klein, 1969). *Eucalyptus viminalis* Labill, an exotic tree from Australia, has a high economic value for the pulp and paper industries (Maluf, 1998).

The use of *Trametes villosa* in the biopulping and bleaching of wood of *Eucalyptus viminalis* (one of the most important sources of cellulose on Southern Brazil) instead of chemical products, could generate a friendly technology for the cellulose industries.

Our main objective was to determine the *in vitro* degradation capacity of *T. villosa,* strain K10, for *E. viminalis* and *M. rostrata*. Additionally, we aimed to establish the white rot type (simultaneous or selective) and to compare the wood strengths of the two species tested.

Material and Methods

Fungus: *Trametes villosa* (Fr.) Kreisel (Aphyllophorales, Basidiomycetes) strain K10 (Micolab/CCB/UFSC/Brazil) was isolated from basidiomes collected at Santa Catarina Island, Brazil. Stock cultures were maintained on PDA (potato dextrose agar) slants at 4°C and transferred to MEA (malt extract agar) plates from which 2 cm diameter plugs were removed for posterior inoculation.

Wood samples: 2 x 2 x 2 cm wood blocks of *Eucalyptus viminalis* Labill (exotic tree) and *Myrcia rostrata* DC (Brazilian native tree) were used. 135 blocks of each species were incubated for 10 months.

***In vitro* wood decay assays**: Vermiculite-block decay assays were prepared with 60g of vermiculite, 3 wood blocks and 100 ml of water in each glass bottle sterilized at 120°C twice. Blocks were dried at 80°C for 72 hours in an oven and then weighed. The plugs removed from MEA plates were inoculated above wood blocks. The bottle tops were perforated to permit air exchange and were incubated in a chamber inside a greenhouse, with 80% wet control during the time of the experiment, and in sunlight. Every two months, 27 blocks (six control) were withdrawn and cleaned of surface mycelia, and subsequently oven-dried at 80°C until dry weight in order to verify weight losses.

Assay of acid-insoluble lignin (Klason): Determination of Klason lignin (Rocha et al., 1993) was performed on 18 blocks (nine with minor and nine with major weight), corresponding to months 2, 6, and 10. The ratio of the percentages of lignin and weight losses (L/W) was calculated (Worrall et al., 1997).

Results

In order to compare *T. villosa*'s decay on *E. viminalis* and *M. rostrata* during the experiment, the average dry weight loss (expressed as a percentage) was used as the degradation rate. Table 1 shows the dry weight loss, and presents data dispersion corresponding to each sample.

TABLE 1 – Loss of dry weight (%) of *E. viminalis* and *M. rostrata* after incubation with *T. villosa*.

	E. viminalis			*M. rostrata*		
Month	mean %	S	median %	mean %	S	median %
2	4,784	2,219	4,900	2,969	0,406	2,935
4	7,798	4,794	6,742	3,096	1,079	2,896
6	18,103	8,299	17,765	3,956	3,395	2,817
8	23,850	11,313	21,617	3,814	1,090	3,829
10	24,787	10,940	22,120	5,081	2,126	4,742

S = standard deviation

The *E. viminalis* data sets corresponding to months 2 and 4, and 4 and 6 show significant differences in dry weight loss. In months 8 and 10, this difference was not noticeable (Figure 1). With regard to *M. rostrata* (Table 1 and Figure 1) the average rates showed a small variation.

Significant differences were verified when comparing the degradation rates caused by *T. villosa* for both *E. viminalis* and *M. rostrata* during all months of analysis. When compared to *E. viminalis*, *M. rostrata* presented an average degradation rate (in percentage) which was considerably lower.

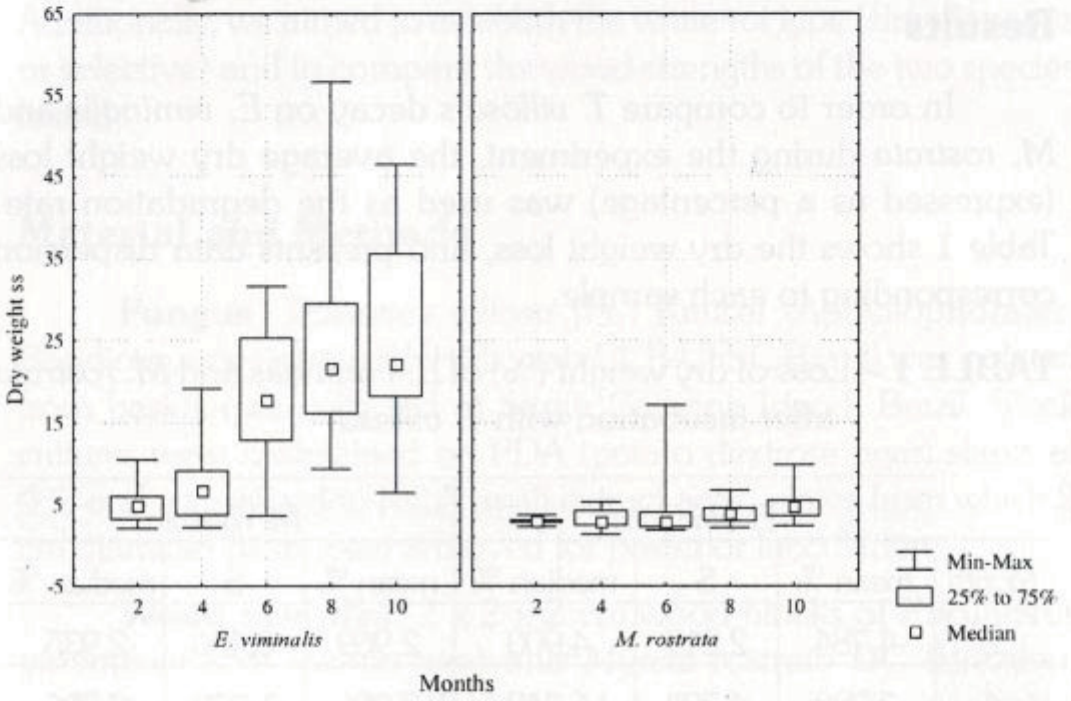

Figure 1: Dry weight loss on *E. viminalis* and *M. rostrata* by the action of *T. villosa*

The highest rate of Klason lignin loss in blocks of *E. viminalis* and *M. rostrata* was registered at the end of the second month. The ratio between the Klason lignin loss (L) and dry weight loss (W) was used to establish the type of degradation caused by *T. villosa* (Worrall et al., 1997). The L/W ratio was higher in the first two months for *E. viminalis* and *M. rostrata* (Table 2).

TABLE 2 – Ratio between Klason lignin loss (L) and dry weight loss (W).

Tree species	Months	W (%)	L (%)	L/W
E. viminalis	2	8,749	9,025	1,032
	6	30,484	2,778	0,091
	10	43,986	4,167	0,095
M. rostrata	2	3,587	11,803	3,290
	6	10,431	7,638	0,732
	10	9,342	8,330	0,892

* Three most degraded samples of each tree species for each month analysed.

Discussion

Data regarding the degradation rate obtained in this work showed that *M. rostrata* wood blocks were relatively rapidly decomposed by *T. villosa* during the first two months, and that this rate remained stable during following months. This rapid degradation, in the first months, was also found by Levin and Castro (1998) when they quantified the degradation rate of wood blocks of *Populus* sp. and *Salix* sp. (Salicaceae) by *Trametes trogii* Berk. These authors also noted a slow degradation rate between months 2 and 4, which we also observed in our data from *E. viminalis*.

Loss of dry weight caused by *T. villosa* did not reach 25% in *E. viminalis* wood and did not exceed 5% in *M. rostrata* wood. These values are relatively low when compared with results obtained with *T. trogii* versus *Salix* sp. and *Populus* sp. (Levin and Castro, 1998) and *T. versicolor* versus *E. viminalis* and *Pinus radiata* (Ferraz et al., 2000). It is possible to relate this degradative capacity with the wood block dimensions, because larger blocks present lower weight loss, probably due to difficulties in accessing and decomposing the inner structure of the wood.

The higher L/W ratio in the first two months for both tree species allowed us to confirm that higher values of dry weight loss (W) correspond to lower values of L/W. However, an increase in dry weight loss does not necessarily imply an increase in Klason lignin loss as Pérez et al. (1993) affirmed for *T. versicolor*. During the action of the white-rot fungi, the delignification is more selective at low weight loss. Considering that the fungi presenting L/W greater than 1 cause selective degradation of lignin (Worrall et al., 1997), we can state that *T. villosa* caused a selective decay of lignin in the two tree species we tested during the first two months. After the second month, the L/W was lower than 1 for both species, which leads us to the conclusion that *T. villosa* acted as a simultaneous white-rot during the following 8 months.

Some fungi appear to attack wood either selectively or non-selectively (simultaneously), producing both types of degradation in the same substrate (Homolka et al., 1994, Blanchette, 1995). Our experiments confirmed that *T. villosa* produces both types of lignin degradation in *E. viminalis* and *M. rostrata*, as already observed by Adaskaveg and Gilbertson (1986) for *Ganoderma* species.

However, we could affirm that *T. villosa* acts diversely on the two tree species. *Eucalyptus viminalis* had a greater decomposition rate when compared to *M. rostrata*, showing that this native species was more resistant to *T. villosa* action.

The white-rot degradation rate is more affected by the quality and composition of the lignin than by its quantity (Eaton and Hale, 1993; Blanchette, 1995). Harsh and Tiwari (1990) believe that the wood structure possibly restricts the quantity of one component available to degrading enzymes and prevents other components from being degraded. Thus, the variable rate and extension of degradation caused by *T. villosa* were possibly influenced by the heterogeneity of the lignocellulotic matrix of the tree species.

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